of equimolar concentration of Zn^{2+} ions formed amyloid like aggregates as observed by secondary structural changes by CD and ability to bind amyloid specific dyes such as congo red and thioflavin T. These aggregates showed fibril-like morphology observed under TEM. The role of Zn^{2+} ions in GH aggregation was further probed by NMR analysis.The involvement of Zn^{2+} in amyloid formation is confirmed as incubation of GH in presence of Zn^{2+} ions and EDTA didn't result in any amyloid formation. Additionally GH fibrils formed with Zn2+ ions destabilizes when incubated with EDTA and results in release of monomers. This data suggests possible mechanism of GH storage and release in somatotrophs of anterior pituitary which could further help in understanding GH deficiency caused due to faults in storage of GH in secretory granules.

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Structural Studies of Betaine Homocysteine Methyl Transferase (BHMT) and a Dimeric Mutant by Conventional and 2DCOS Moving Lapse IR Spectroscopy

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Betaine homocysteine S-methyltransferase (BHMT, EC 2.1.1.5) is one of the two enzymes known to methylate homocysteine (Hcy) to generate methionine in the liver. The increase in plasma levels of Hcy (homocysteine) has been established as an independent risk factor for cardiovascular and Alzheimer diseases. BHMT uses betaine as the methyl donor to synthesize methionine, allowing recovery of one of the methyl groups used in choline synthesis by transmethylation. Changes in BHMT activity have been detected under several dietary conditions, during development and in pathologies such as cirrhosis and hepatocellular carcinoma. Rat liver BHMT is a 407-aminoacid cytosolic protein that is more than 90% identical at the amino acid level with its human and pig counterparts. The enzyme contains zinc co-ordinated to three conserved cysteine residues and assembles as a homotetramer. BHMT possesses seven tryptophan residues per subunit located along the sequence including the dimerization arm. Also they are implied in the dimerization arm. We have used a conventional and 2DCOS infrared approach to make a structural study of the differences between BHMT and the dimeric W325F mutant. In these studies, temperature has been used as perturbation. We have also used to extract information from the 2DCOS maps a ''moving lapse'' approach which uses a narrow frame of 2D spectra along perturbation in order to highlight local variations in structure instead of attending global changes. The mutant has a different amide I shape with a decrease in the area percentages of α-helix and an increase in the band at 1624 cm-1 attributed to extended structures. The moving lapse approach shows the sequence of changes and the structural difference between BHMT and the mutant.

DNA Replication, Recombination, and Repair

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Studies of DNA Gyrase at the Single Molecule Level

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Gyrase, a bacterial type II topoisomerase, is the only topoisomerase capable of introducing negative supercoils into DNA. Negative supercoiling results from the coordinated movement of double stranded DNA and protein domains linked to ATP hydrolysis. Thus, it is important to combine structural data with dynamic information of protein and/or DNA movements to obtain a comprehensive picture of the mechanism of gyrase. Although the structures of several individual domains of different type II enzymes have been elucidated, there are still no structures of the intact gyrase heterotetramer or an intact gyrase/DNA complex precluding the correlation of conformational changes in the protein with movements of DNA. To alleviate this shortcoming, we are combining structural and single molecule studies to investigate the conformational changes that occur in gyrase as it alters DNA topology. To facilitate our research, we have optimized a procedure for purifying stable gyrase/DNA complexes in the amounts required for biophysical studies. This method results in homogeneous complexes that are captured in well-defined states along the catalytic cycle. We are also developing a novel single molecule technique combining magnetic tweezers and fluorescence microscopy to study the movements of both protein and DNA during the DNA supercoiling process. The combination of dynamic single molecule and structural information promises to provide a more comprehensive picture of the mechanism used by this molecular machine to alter DNA topology.

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Real Time Visualization of hRPA Binding to Torsionally Controlled Double-Stranded DNA

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In eukaryotic cells, single-stranded DNA (ssDNA) is rapidly bound and stabilized by ssDNA-binding proteins (SSBs). This prevents ssDNA from binding back on itself into secondary structures. The main eukaryotic SSB is replication protein A (RPA), which is important for repair, replication and recombination. We study the dynamics of human RPA (hRPA) on topologically constrained DNA at the single-molecule level with magnetic tweezers. This assay allows us to apply varying torsional stress and stretching forces on the dsDNA, parameters that are known to influence the hRPA unwinding reaction. We are interested in uncovering positional preference, cooperativity and directionality of hRPA-DNA binding. To observe this directly, we visualize the position(s) of the hRPA along the DNA, using a combination of magnetic tweezers with fluorescence microscopy. With magnetic tweezers a dsDNA molecule is held between a glass surface and a magnetic bead by a pair of magnets. The stretching force and torsional stress is controlled by the distance and rotations of the magnets. The molecule is pulled sideways and the fluorescently labeled RPA is imaged with an inverted microscope. By applying negative supercoiling at high forces, bubbles of ssDNA open up. Multiple bubbles will allow RPA to bind to multiple sites, and such RPA-stabilized bubbles may coexist or slowly anneal into one larger bubble. Our first experiments show that more than one RPA-binding sites occurs when assembled on 20 kb dsDNA that is underwound. These RPA-bound spots appear to be stable in time. We will report the structure and dynamics of RPA-stabilized ssDNA bubbles at the meeting.

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Single Molecule Observation of Direct Transfer of Escherichia Coli Single-Strand Binding Protein (SSB) between Single-Stranded DNA Molecules

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Echerichia coli Single-Strand Binding (SSB) protein is essential in DNA replication and repair processes owing to its ability to bind the intermediate single stranded DNA (ssDNA), thereby preventing unwanted reannealing or degradation. It was proposed that SSB is recycled by redistributing itself along long ssDNA, for example between two adjacent Okazaki fragments. The mechanism of this process was investigated by the Lohman group using ensemble kinetics methods and they proposed a ''direct transfer'' mechanism - forming a transient intermediate composed of SSB and two ssDNA prior to the transfer. We developed a single molecule assay to examine this direct transfer in detail through real-time observation of single molecule fluorescence resonance energy transfer (smFRET) signals. The introduction of competitor ssDNA oligonucleotides to an SSB protein bound to a surface immobilized ssDNA ultimately led to the dissociation of SSB from the surface immobilized DNA. The rate of SSB transfer is linearly dependent on the competitor DNA concentration up to 50 nM, suggesting that only one ssDNA molecule is involved in the rate-determining step of the transfer process. Prior to the full unwrapping of the original DNA bound to an SSB protein, fast FRET fluctuations with 100 ms time scale were observed with the distribution of the FRET signal shifted toward the lower value, indicating the structural destabilization of the ssDNA-SSB complex induced by multiple events of competitor ssDNA binding. The final dissociation event measured as a high to low FRET transition occurs rapidly. These findings are consistent with the kinetic model_short-lived, partially unwrapped, intermediate states were induced by the fast binding and unbinding of competitor oligos and only a small fraction of the intermediate state molecules can achieve complete transfer of SSB.

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Structural Studies of Rolling Circle Replication Initiator Proteins

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pT181 family plasmids replicate by a rolling-circle mechanism. This is initiated by a plasmid-encoded Rep initiator protein, which has sequence-specific DNA nicking and religation activity. The replication origin is nicked by Rep, which binds covalently to one DNA strand via an active site tyrosine, initiating rolling circle replication and religating the strand at the end of the cycle. Rep proteins also associate with PcrA helicase to form a highly processive complex. We